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ESTERASES AND NEUROTOXICITY OF SOME ORGANOPHOSPHORUS COMPOUNDS

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Abstract—The inhibition by organophosphorus compounds *in vivo* of two esterases present in chicken brain and spinal cord (phenyl phenyl-acetate and phenyl 3-phenyl-propionate as substrates) has been studied. Although both esterases are inhibited by compounds which cause ataxia in chickens, they are also inhibited by those which do not. Therefore, inhibition of these esterases is unlikely to be involved in the production of the lesion in the chicken central nervous system.

THE MAJORITY of organophosphorus compounds do not cause the neurotoxicity in domestic fowls characterized by the development, at least 8 days after dosing, of ataxia progressing to paralysis of the limbs. Nevertheless the number of groups of organophosphorus compounds which have this property is increasing.[1] Amongst them the only apparent common characteristics are that they all contain phosphorus and (either directly or indirectly after metabolism in the fowl) have the property of inhibiting enzymes with esteratic activity. Since neither of the cholinesterases of the central nervous system appear to be involved,[8, 19, 20] other esterases may be implicated.

The reaction of organophosphorus compounds with esterases is in principle the same type of process as the hydrolysis of substrates.[2] Phenyl phenylacetate and phenyl 3-phenylpropionate, which resemble structurally the highly neurotoxic phenylsaligenin phosphates,[3] are rapidly hydrolysed by enzymes present in chicken brain, spinal cord and sciatic nerve.[4] Two esterases which hydrolyse these esters have been characterized and methods devised for their determination.[5] This paper describes the results of measurements of the activity of these esterases in the brain and spinal cord of chickens at various times after the administration of both neurotoxic or non-neurotoxic organophosphorus compounds.

METHODS

Determination of esterase activity

The methods used for the preparation of the homogenates and the manometric determination of esterase activity using phenyl phenylacetate and phenyl 3-phenyl-propionate have been described previously.[4, 5] The activity of the two major esterases have been calculated from these results.[5] Esterase X has a ratio of hydrolysis of phenyl 3-phenylpropionate to phenyl phenylacetate (ratio P/A) of 0·05 and its activity is given as a rate of hydrolysis of phenyl phenylacetate. Esterase Y has a ratio P/A of 9·75 and its activity is given as a rate of hydrolysis of phenyl 3-phenylpropionate.

TABLE 1. ROUTE AND METHOD OF ADMINISTRATION OF ORGANOPHOSPHORUS COMPOUNDS TO CHICKENS

Compound	Dose/kg	Injection medium	Other treatment	Ataxia (references)
Tri 4-methyl phenyl phosphate	0·5 g oral	Arachis oil	—	−6–9
Tri 2-methyl phenyl phosphate	1·0 ml oral	Undiluted compound injected	—	+8–10
Tri 2-ethyl phenyl phosphate	1·0 ml oral	Unduluted compound injected	—	−9,10
Mono 2-methylphenyl diphenyl phosphate	0·1 g oral	Undiluted compound injected	—	+1,11
Phenyl saligenin phosphate	5 mg i.p.	Arachis oil	—	+1,12
Diethyl *p*-nitrophenyl phosphate	2 mg s.c.	Ethanol	*	−13
Di isopropyl phosphorofluoridate	2 mg s.c.	Ethanol	*	+13–15

i.p. intraperitoneal; s.c. subcutaneous.

* Additional prophylactic treatment with 5 mg/kg atropine sulphate and 50 mg/kg pyridine 2 aldoxime methane sulphonate.

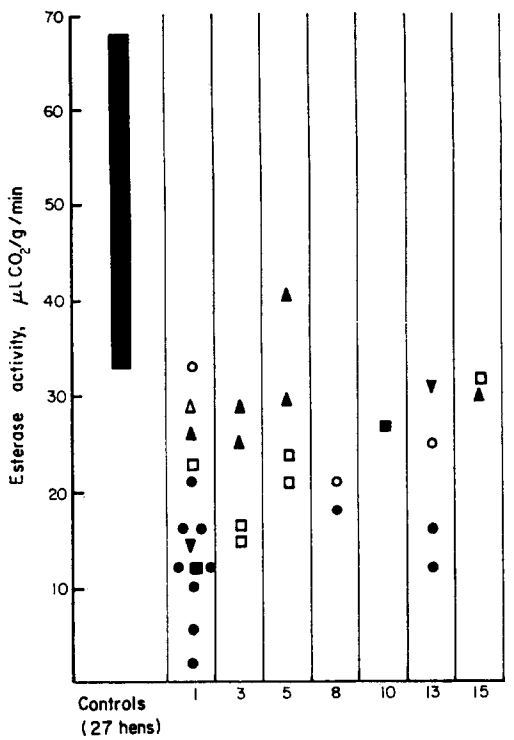

FIG. 1. The inhibition of Esterase X of chicken brain by various organophosphorus compounds. The activity is expressed in terms of the hydrolysis of phenyl phenylacetate. The filled symbols are those compounds which produce ataxia in chickens and the open symbols are those which do not. ●, tri 2-methylphenyl phosphate; ▲, di isopropyl phosphorofluoridate; ■, phenyl saligenin phosphate; ▼, mono 2-methylphenyl diphenyl phosphate; ○, tri 2-ethylphenyl phosphate; △, tri 4-methylphenyl phosphate; □, diethyl *p*-nitrophenyl phosphate.

Animals and methods of administration of organophosphorus compounds

All chickens were between 6 and 12 months of age. Due to the different physical and chemical characteristics of the compounds they were administered by different routes and in different media. The exact procedure is given in Table 1 together with references to their activity in producing ataxia in chickens.

RESULTS AND DISCUSSION

At the various times after the administration of organophosphorus compounds to the chickens the activity of esterases X and Y have been determined in brain and spinal cord. The results are given in Figs. 1–4.

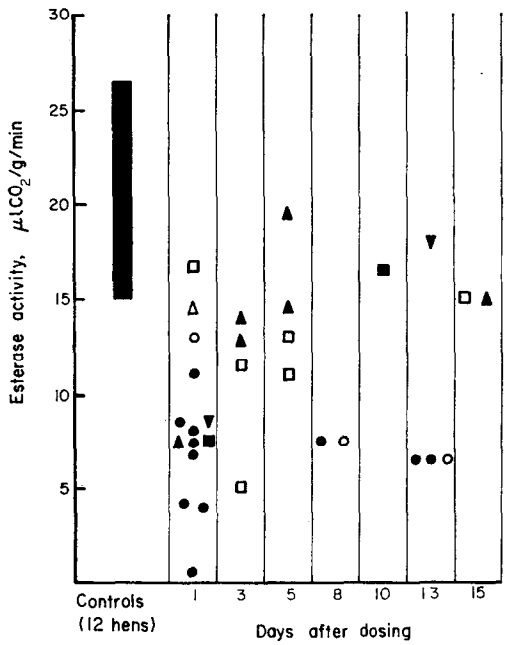

FIG. 2. The inhibition of Esterase X of chicken spinal cord by various organophosphorus compounds. The activity is expressed in terms of the hydrolysis of phenyl phenylacetate. The filled symbols are those compounds which produce ataxia in chickens and the open symbols are those which do not ●, tri 2-methylphenyl phosphate; ▲, di iospropyl phosphorofluoridate; ■, phenyl saligenin phosphate; ▼, mono 2-methylphenyl diphenyl phosphate; ○, tri 2-ethylphenylphosphate; △, tri 4-methylphenyl phosphate; □, diethyl *p*-nitrophenyl phosphate.

Compounds which produce ataxia do inhibit esterases X and Y but inhibition is also found after the administration of organophosphorus compounds which do not produce ataxia, e.g. diethyl *p*-nitrophenyl phosphate[13] and tri(2-ethylphenyl)-phosphate.[8, 9] After the administration of organophosphorus compounds there is always a latent period of at least 8 days before the appearance of clinical signs. There is however no difference in the persistence of inhibition of either esterase X or Y after administration of neurotoxic and non-neurotoxic compounds.

The inhibition of pseudo-cholinesterase of the central nervous system has previously been considered to be involved in the production of lesion in chickens.[16–18] This possibility has also been eliminated by procedures similar to those used in this paper, i.e. the demonstration that inhibition is obtained by compounds which do not cause ataxia.[8, 19, 20] Such a comparison depends upon an assumption that the organophosphorus compounds which induce this lesion do so by an identical biochemical lesion. Direct evidence for this is, of course, not available but such features as the time between the dose and the appearance of the ataxia and the age when chickens become sensitive as well as the pathological lesion are identical.

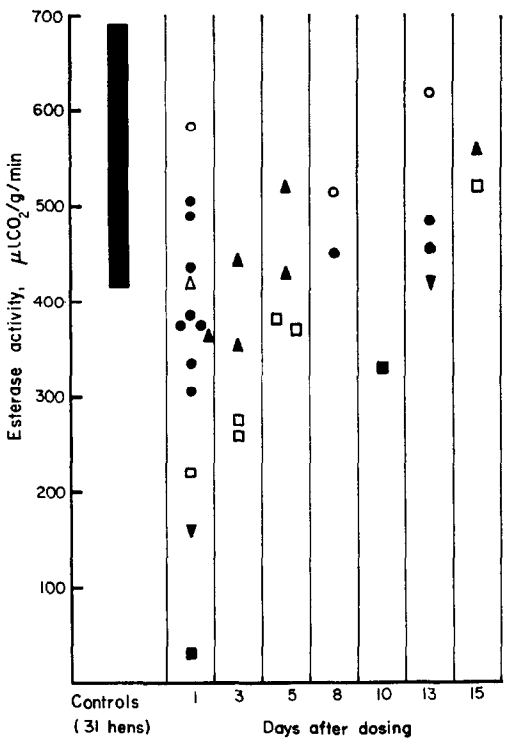

FIG. 3. The inhibition of Esterase Y of chicken brain by various organophosphorus compounds. The activity is expressed in terms of the hydrolysis of phenyl 3-phenylpropionate. The filled symbols are those compounds which produce ataxia in chickens and the open symbols are those which do not. ●, tri 2-methylphenyl phosphate; ▲, di isopropyl phosphorofluoridate; ■, phenyl saligenin phosphate; ▼, mono 2-methylphenyl diphenyl phosphate; ○, tri 2-ethylphenyl-phosphate; △, tri 4-methylphenyl phosphate; □, diethyl *p*-nitrophenyl phosphate.

It seems unlikely therefore that neither pseudo-cholinesterase, nor esterases X and Y can be involved in the production of the neurological lesion. Nevertheless, all of the increasing number of organophosphorus compounds which have this neurotoxic activity are powerful inhibitors of esterases or are converted by the chicken to active inhibitors. It is, therefore, still a tenable hypothesis that the inhibition of an esterase

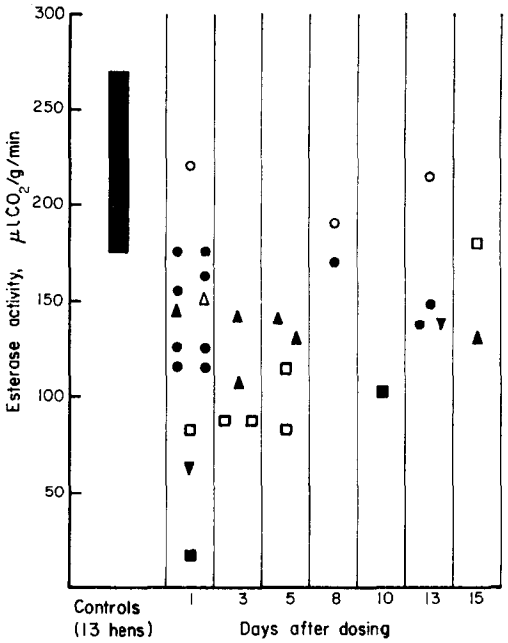

FIG. 4. The inhibition of Esterase Y of chicken spinal cord by various organophosphorus compounds. The activity is expressed in terms of the hydrolysis of phenyl 3-phenylpropionate. The filled symbols are those compounds which produce ataxia in chickens and the open symbols are those which do not. ●, tri 2-methylphenyl phosphate; ▲, di isopropyl phosphorofluoridate; ■, phenyl saligenin phosphate;▼, mono 2-methylphenyl diphenyl phosphate; ○, tri 2-ethylphenyl-phosphate; △, tri 4-methylphenyl phosphate; □, diethyl *p*-nitrophenyl phosphate.

is involved in the development of the lesion. The analytical techniques used in this study[5] cannot, however, detect small amounts of other esterases which we know exist in the chicken central nervous system. These must be examined but they present formidable analytical problems.

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